Abstract

This thesis deals with barriers and factors critical for development of viruses, leishmania and gregarines in sand flies.

First, we focused on life cycle of sand fly-borne phleboviruses, especially possible routes of sand fly infection. As a laboratory model we chose Massilia virus (MASV), species closely related to Toscana virus, which is main causative agent of summer meningitis in Mediterranean area. We tested different ways of infection by MASV in various developmental stages of *Phlebotomus perniciosus*; infection of (i) first (L1) and fourth (L4) instar larvae through larval food, (ii) females by blood meal, (iii) both sexes by sugar meal. Infection of L1 and L4 by larval food and subsequent transstadial MASV transmission to adults were not efficient; from 875 adults only three were MASV-positive. Infection through bloodmeal led to high infection rate before defecation, nevertheless, post defecation the infection rate declined and only 5 out of 27 females were MASV-positive. The most efficient infection way was through the sugar meal: 72% of females (88 out of 122) and 51% of males (58 out of 113) were detected as MASV-positive. Moreover, both males and females infected by this way released MASV particles into the drop of sugar which stayed infectious for next 24 hours for other naïve sand flies; almost 30% *P. perniciosus* became infected after feeding on this sugar with regurgitated virus. We suppose that common feeding of infected and uninfected sand flies on the same sugar meal could be important part of *Phlebovirus* circulation in the nature.

Sand flies are well-known vectors of leishmania and sand fly peritrophic matrix (PM) was proposed as important barrier for leishmania development in some sand fly species, especially *Sergentomyia schwetzi.* We experimentally confirmed this theory by addition of *Beauveria bassiana* chitinase into infectious bloodmeal of *S. schwetzi*. In chitinase-treated *S. schwetzi* the PM was disrupted earlier and *Leishmania major* and *Leishmania donovani* had enough time to escape into ectoperithrophic space and develop mature infection with metacyclic forms and colonization of the stomodeal valve. In control group, no leishmania were able to survive defecation of bloodmeal remnants and all infections were lost.

As shown in mosquitoes, ambient temperature during both larval and adult life affects vector competence. We tested impact of different larval rearing temperature (27°C and 32°C) on susceptibility of *Phlebotomus sergenti* females to *Leishmania tropica*. Larvae kept at higher temperature developed faster and produced smaller females, nevertheless infection rate or intensity of infection *L. tropica* did not differ between groups maintained at different temperature. Interestingly, increase of temperature during larval development eliminated gregarines *Psychodiella sergenti*; all sand flies emerged from larvae and pupae maintained at 27°C were infected with

gregarines, with the mean number of gamonts per individual 29.5. In contrast, only in three adults out of 120 developed from larvae and pupae kept at 32°C were found positive for gregarines.

Finally, leishmania and gregarines may naturally co-occur in sand flies. In mosquitoes it was shown that the presence of gregarines affects development of other pathogens. Therefore, we decided to test whether the presence of gregarine *Ps. sergenti* in sand flies *P. sergenti* affects development of *L. tropica*. However, we did not find any significant differences in intensity of infection and infection rate of *L. tropica* between females infected and non-infected by gregarines.